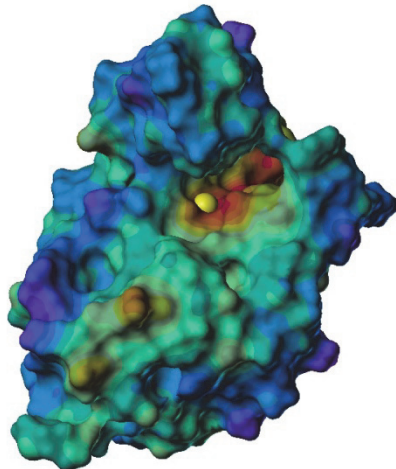


B



C

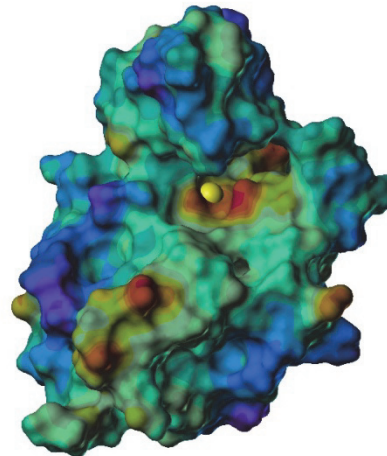


Figure S1. PlyP56 alignment with structural homolog Ply500. (A) Sequence alignment of *L. monocytogenes* phage A500 (Ply500) from PDB entry 2VO9 and PlyP56 EADs (Clustal X alignment symbols: asterisk = identical; colon = strongly similar; period = weakly similar; space = not similar). Overall percent identity ($\# \text{ identical} / \# \text{ total}$) = 70.1%; percent similarity [$(\# \text{ identical} + \# \text{ strongly similar}) / \# \text{ total}$] = 81.6%. Arrows indicate the metal-binding residues (red) and the catalytic base/acid (blue). The conserved SxHxxGxAxD zinc-binding motif is highlighted with a red rectangle. Colored ovals represent sequence insertions or deletions; see Fig. 5. (B–C) Connolly surfaces color-coded by electrostatic potential (blue = most positive; red = most negative) for the template Ply500 (B) and the modeled PlyP56 (C) EAD; a yellow sphere represents the Zn^{2+} ion.

A

			10	20	30					
1yb0_a	1	MEIRK	KL	VVP	SKYGT	KCPYT	MKPKY	ITVHN	30	
PlyN74	1	MNINT	QYLV	T	DPERL	KVINW	MNPTE	ITFHN	30	
		:	..:	:*	.	*	*:*	**:*		
			40	50	60					
1yb0_a	31	TYNDAP	AE	NE	VNYMI	TNNNE	VSFH	VA	DDDK	60
PlyN74	31	TYNDAS	ASAE		VRNVR	RNNSTG	TSFH	TA	VDDF	60
		*****	.*	*	*.	:	.*	..	***.*	****
			70	80	90					
1yb0_a	61	QAIQG	IP	WER	NAWAC	GDGN-	GPGN	RES	SISV	89
PlyN74	61	EVQQV	VP	FDR	NAWHAG	DGTY	GAGNR	NS	IGV	90
		..*	:	***:	*	***.	*	***:	**.*	
			100	110	120					
1yb0_a	90	EICYS	KS	GGD	RYYKA	ENNAV	DVVR	QL	MSMY	119
PlyN74	91	EICYS	MS	GGE	RYRKA	EELNAI	EHIS	DL	LMVRF	120
		*****	***:	**	***	**:	:	:	**	:
			130	140	150					
1yb0_a	120	NIPIE	NR	TH	QSWSG	KYCPH	RMLAE	GR	WGWA	149
PlyN74	121	GIPIS	KV	KTH	QERN	GKYCPH	RMLDE	GR	VGVW	150
		.*	..:	**:	*	..*	*****	***	***	*
			150	155						
1yb0_a	150	FIQK	VK				155			
PlyN74	151	FKAE	CE				156			
		*	:	:						

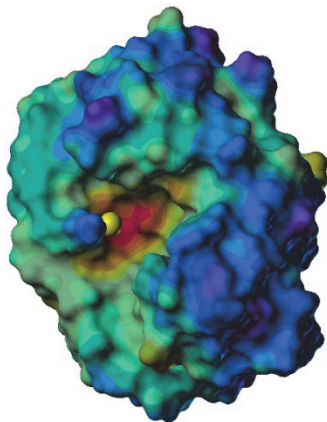
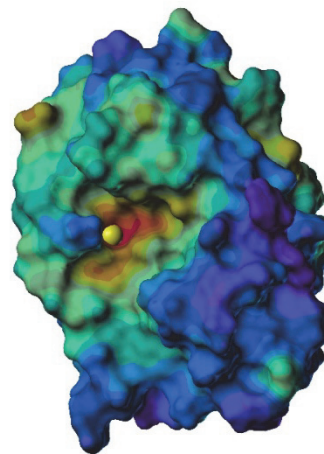
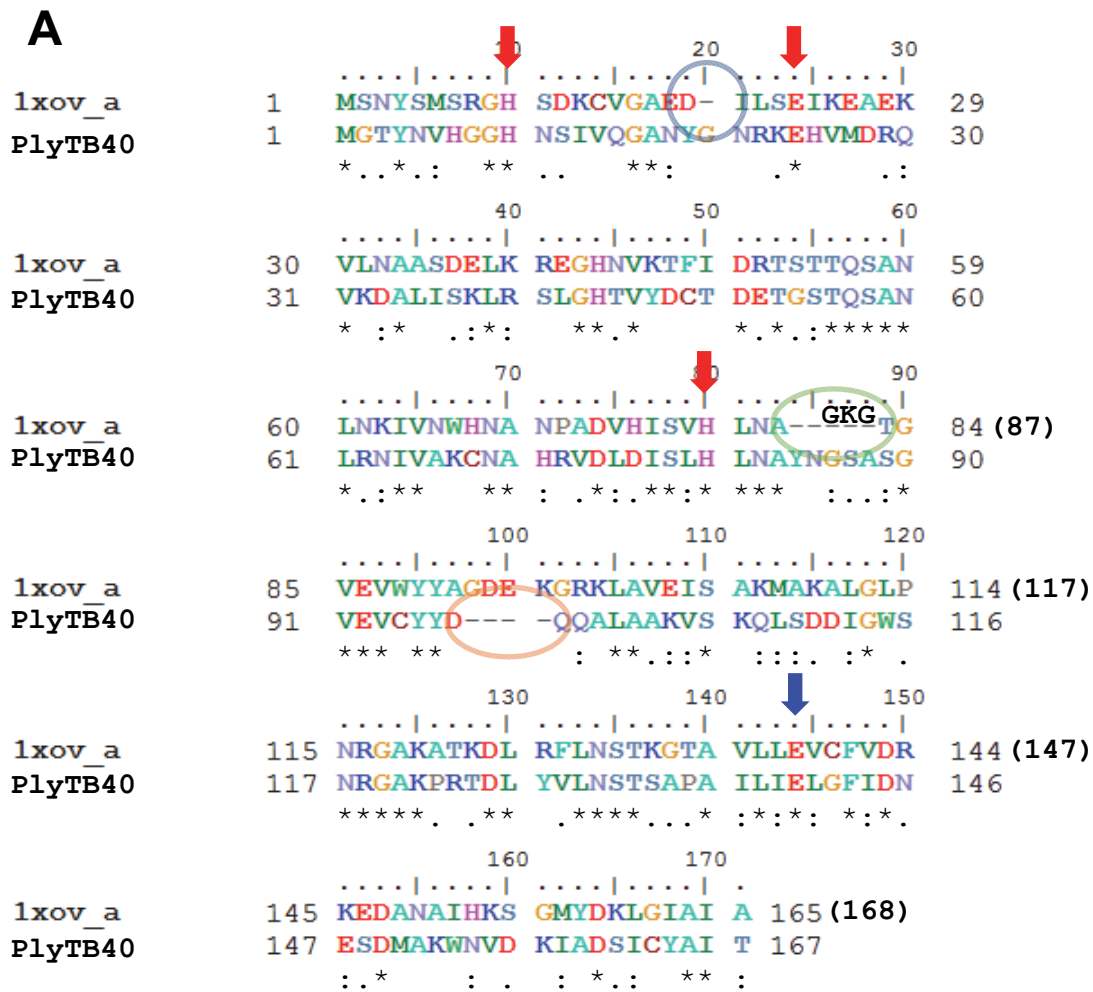
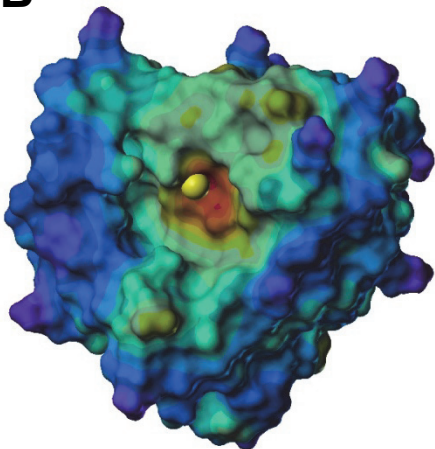
B**C**

Figure S2. PlyN74 alignment with structural homolog PlyL. (A) Sequence alignment of *B. anthracis* λ prophage Ba02 (PlyL) from PDB entry 1YB0 and PlyN74 EADs (Clustal X alignment symbols: asterisk = identical; colon = strongly similar; period = weakly similar; space = not similar). Overall percent identity ($\#$ identical / $\#$ total) = 51.3%; percent similarity [$(\#$ identical + $\#$ strongly similar) / $\#$ total] = 64.1%. Arrows indicate the metal-binding residues (red) and the catalytic base/acid (blue). Colored ovals represent sequence insertions or deletions; see Fig. 5. (B–C) Connolly surfaces color-coded by electrostatic potential (blue = most positive; red = most negative) for the template PlyL (B) and the modeled PlyN74 (C) EAD; a yellow sphere represents the Zn^{2+} ion.



B



C

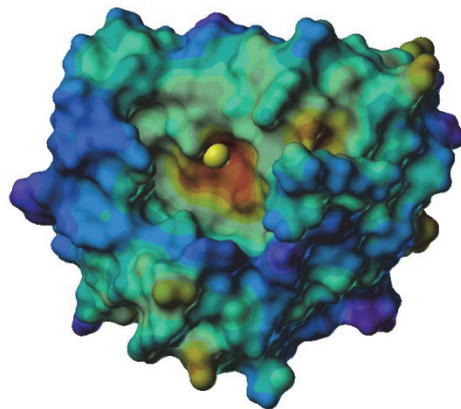


Figure S3. PlyTB40 alignment with structural homolog PlyPSA. (A) Sequence alignment of *L. monocytogenes* phage PSA (PlyPSA) from PDB entry 1XOV and PlyTB40 EADs (Clustal X alignment symbols: asterisk = identical; colon = strongly similar; period = weakly similar; space = not similar). Overall percent identity (# identical / # total) = 36.3%; percent similarity [(# identical + # strongly similar) / # total] = 53.2%. Arrows indicate the metal-binding residues (red) and the catalytic base/acid (blue). Colored ovals represent sequence insertions or deletions; see Fig. 5. (B–C) Connolly surfaces color-coded by electrostatic potential (blue = most positive; red = most negative) for the template PlyPSA (B) and the modeled PlyTB40 (C) EAD; a yellow sphere represents the Zn²⁺ ion.

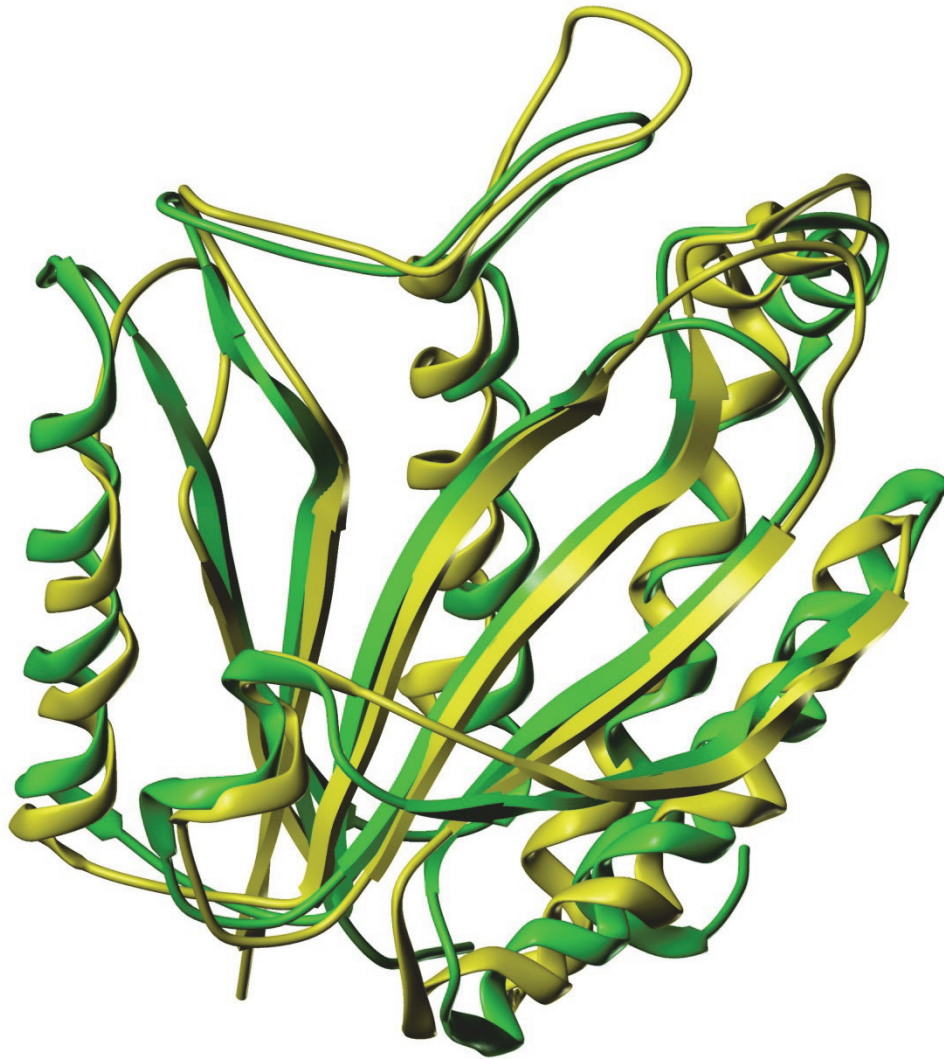


Figure S4. Carboxypeptidase T-type Amidase_3 fold. Ribbon diagrams of the endolysin EADs of *Listeria monocytogenes* bacteriophage PlyPSA (PDB ID: 1XOV; PlyTB40 homology modeling template; green) and *Bacillus polymyxa* var. *colistinus* CwIV (PDB ID: 1JWQ; yellow), each exhibiting the carboxypeptidase T-type Amidase_3 fold.

Supplementary Material

Table S1: Primers used in this study

Template	Primer	Sequence (5' > 3')*
pBAD24:: <i>plyP56</i>	IR29 F'	CGT <u>GAATTC</u> CATGCATCATCATCATCATGATTATGATA GCAGCTGG
	IR30 R'	CGT <u>TCTAGAT</u> TATTTAAACGTGCCCCAATA
pBAD24:: <i>plyN74</i>	IR33 F'	CGT <u>GAATTC</u> CATGCATCATCATCATCATGATTATGATA GCAGCTGGTTTACC
	IR34 R'	CGT <u>TCTAGAT</u> TATTTAAAGGTGCCCCAATAATTCAC
pBAD24:: <i>plyTB40</i>	IR37 F'	CGT <u>GAATTC</u> CATGCATCATCATCATCATCATTATGATAGCA GCTGGTTTACACCG
	IR38 R'	CGT <u>TCTAGAT</u> TACTGAAAGGTGCCCCAATAGCTAAT

* Restriction sites underlined